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A Cortisol Study; Facial Hair and Nails

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Abstract

Current methods for measuring cortisol levels can be challenging due to the need to take multiple urine, saliva or serum samples. Therefore, it seems necessary to find alternative matrices which can be used as stress indicators in which sample collection methods are non-invasive. Two experiments were conducted to first to test the feasibility of cortisol levels in facial hair and second to find a correlation among facial hair cortisol and cortisol levels in nails. In the first experiment, facial hair from five subjects was analyzed to confirm the presence of cortisol. The results of the assessment of facial hair showed that facial hair may be used to measure cortisol levels over a short period of time. In the second experiment, nineteen university students (males who regularly partake in martial arts- aged: 22 ± 3.15) provided fingernail, toenail and facial hair samples at set intervals throughout the school year; 1) The Study Period- during the student's every-day life (minimal stress conditions, baseline), 2) The Exam Period- following student's final exams (mental stress), and 3) The Fighting Period- following intensive martial arts training (physical stress). Cortisol in facial hair, toenails, and fingernails showed higher levels during both the Exam Period and the Fighting Period when compared to the baseline Study Period ($p<0.05$). However, no differences in cortisol levels were observed among the indices during the Exam Period and Fighting Period ($p>0.05$). A higher correlation in cortisol levels was observed between facial hair samples and toenails ($r=0.73$) than between fingernails and toenails ($r=0.61$). Overall, cortisol levels showed significant correlations between fingernails and toenails ($p=0.001$, $r=0.61$), fingernails and facial hair ($p=0.01$, $r=0.54$), and toenails and facial hair ($p=0.001$, $r=0.73$). Further research is needed to understand the relationship between facial hair and nail cortisol and their possible relationship with health disorders.

Keywords: Cortisol; Facial hair; Finger nails; Stress; Toe nails

Background

In recent years, the scientific community's interest in stress related cortisol has increased dramatically, along with the stresses of Westernized day-to day life. The effects of prolonged exposure to stress in our daily routines results in what we call allostatic load. When there is an increase in the allostatic load, it can result in receptor desensitization, tissue damage, and other physical and mental maladies [1]. For these reasons and more, it is of vital importance to accurately measure short term cortisol levels caused by both mental and physical stress. Under conditions of physical and psychological stress [2] the cortisol hormone is released by the zona fasciculate of the adrenal cortex in the kidneys and periphery- which is stimulated by an HPA-like axis (Hypothalamus Pituitary Axis) within the hair follicle [3,4]. Moreover, it is well documented that blood-borne substances are able to diffuse from capillaries into the cells of hair follicles and subsequently become deposited into the hair shaft [5] and fingernails [6].

Recently it has been hypothesized that cortisol is incorporated into both hair and nails via similar cellular mechanisms [7]. In this study, we build upon this hypothesis, theorizing that cortisol within facial hair is incorporated in the same fashion. Saliva and serum cortisol levels are subject to major physiological circadian fluctuations. Additionally, the collection method used with these mediums is invasive and costly. Alternatively, hair and nail analysis presents a longer history of cortisol levels, as well as a non-invasive, cost effective means of collecting samples.

A pilot study by Warnock et al. [6] was the first to find cortisol in finger nails. The data showed the feasibility of cortisol assessment in fingernails and significant correlations between cortisol and DHEA in subjects. A follow-up study done by Izawa et al. [8] confirmed these results. Yet, to date, no existing study has investigated potential variations in cortisol levels between fingernails and toenails.

Current data shows hair cortisol levels vary across the body's different regions [4] such as between the scalp and legs. Notably, this study does not mention facial hair. To the best of the author's knowledge, there has been no research investigating cortisol levels in facial hair, possible variations between fingernail and toenail cortisol, and correlations between facial hair and nails.

The question of whether cortisol levels show similar trends during the Fighting Period (physical-stress period) and the Exam Period (mental-stress period) remains. Furthermore, the possible incorporation of cortisol into facial hair and toenails in comparison to fingernails needs to be addressed. This study may offer a clearer

understanding of the relationship between accumulated cortisol in body end-points (facial hair and nails), and how these levels may differ in subjects while under mental and physical stress.

Materials and Methods

Participants

Samples were collected from subjects at three distinct periods; 1) The Study Period (minimal stress, baseline), 2) The Exam Period (mental stress), and 3) The Fighting Period (physical stress). Participants were asked to complete a short questionnaire regarding the subject's general health (age, smoking status and medication intake). Baseline data during each period was used as a control group to eliminate other potential confounding factors while under stress conditions. Furthermore, logs of training frequency (training hours per week in the previous 3 months) and study stress (perceived stress during exam preparation) were compiled. The Perceived Stress Scale [9] was used to assess overall perceived stress.

Materials and sample collections from participants

To test for the presence of cortisol in facial hair, five subjects were recruited from the University of Shiraz, Iran to provide facial hair samples prior to the beginning of the experiment (Figure 1). Due to higher sensitivity of the saliva kit (0.002 mg/dL) used in this study; a saliva test was used as a golden standard to validate the obtained data.

Participants for the experimental group were selected for sampling if they met the following eligibility criteria: 1) a sufficient amount of facial hair in the requested time (10 days), 2) no significant physical or mental health problems (based on self-report), 3) currently not taking any medications (including vitamins, minerals and supplements), and 4) 19 years of age or older, 5) participating regularly in 4 or more hours a day of intensive physical training to insure there is an observable amount of physical stress. Of the 29 applicants, 19 male subjects passed the recruitment criteria.

Data was collected at: 1) The Study Period- March 2014, at the beginning of the school semester (baseline), 2) The Exam Period- Late June 2014, at the end of the final exam period (mental stress), 3) The Fighting Period- August 2014, in the middle of the vacation during intensive martial arts training (physical stress). Prior to the beginning of each Period, subjects were asked to clip finger nails and toenails and to shave facial hair. In order to find baseline cortisol levels within samples, samples were collected and measured at the beginning of each Period. At the end of each Period, samples were again collected and cortisol levels were measured to give an accurate comparison between non-stress and stressed conditions. Subjects were then instructed not to trim or wash nails or facial hair for 10 consecutive days. At the end of 10 days, subjects were asked to clip the new growth of nails and facial hair, and place them into separate aluminum foils envelopes. A refresh rate of 2-3 [6] months was allotted for subjects to grow entirely new nails. This was done to insure that no residual cortisol form the previous Period of Stress could contaminate the next Period's test results. Facial hair was shaved using a Philips electric razor (model no. 5053, pivoting and flexing head, Germany). Finger and toe nail samples were provided from every digit. All the procedures of sampling nails and facial hair were approved by the local Clinical Research Ethics Board and Medical School at Hallym University, Chuncheon, South Korea.

Facial hair cortisol extraction and analysis

Facial hair samples from each subject (150 mg) were placed in 5 ml polypropylene tubes. Two ml of iso-propanol were added to each sample twice, totaling in 4 ml of isopropanol. Samples were then gently mixed on a rotator at room temperature for 3 minutes per wash. 1 ml of solvent from each wash was collected in separate tubes to determine the level of overall cortisol lost during the washing procedure. The facial hair samples were then allowed to dry for 7 days in a clean protected hood [10].

After drying, approximately 50 mg of finely ground facial hair samples were weighed and placed into a 2 ml micro centrifuge tube. 1 ml of methanol was added to each micro centrifuge tube, and the tubes were then incubated at room temperature for 24 hours on a slow rotation to extract cortisol.

After the incubation period was completed, 0.6 ml of each micro centrifuge tube was extracted and placed into new micro centrifuge tubes. Then, these 0.6 ml samples were spun for one minute in a micro centrifuge (1000 rpm) before being dried at 38°C under a stream of N_2 gas. The dried extracts were reconstituted with 0.4 ml of phosphate buffer provided in the assay kit for analyzing cortisol levels. The remaining fraction of finely reduced facial hair was dissolved in methanol and was analyzed for cortisol levels.

Facial hair cortisol levels were analyzed using the salivary commercial ELISA test method [11]. The sensitivity was 0.002 mg/dL. All standards and reagents were provided in the kit. All samples were measured during each individual testing period in order to eliminate methodological issues. The salivary ELISA test method provided very precise and consistent data with the lowest standard deviation. The intra-assay and inter-assay coefficients of variations were 1.05 and 9.85, respectively.

Nail cortisol extraction

Isopropanol, 5 ml was used to vortex-wash nail samples for one minute. This process was done twice. The samples were then dried overnight and ground into a powder by a mortar and pestle. The ground nail powder was transferred to a 5 ml polystyrene round-bottom tube and 1 ml of methanol was added. The tubes were then incubated at room temperature for 24 hours on a slow rotation to extract cortisol. The remaining steps were the same as the following facial hair cortisol extraction method.

Statistical analysis

Statistical analysis was carried out using the GLM procedure of SAS (version 9.1; SAS institute Inc., Cary, NC). The GLM procedure was employed and a correlation between variables was tested. To examine correlations between the data sets of toenails, fingernails, and facial hair of subjects, Pearson Product-Moment and Spearman Rank-Order were tested. In order to confirm the correlation results, the data set was analyzed again for correlation analysis using proc CORR.

Results

Data from the preliminary experiment piloted the feasibility of cortisol measurement in facial hair from 5 subjects and is presented in Figure 1.

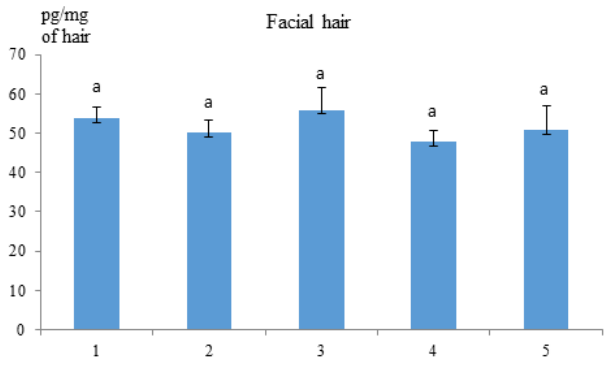

Figure 1: Cortisol levels in facial hair of 5 subjects (preliminary assessment). Five subjects were recruited to provide facial hair samples prior to the beginning of the experiment to test the feasibility of measuring cortisol levels in facial hair

Mean cortisol levels in the fingernail clippings of subjects exposed to mental stress (Exam Period) and physical stress (Fighting Period) conditions were higher ($p<0.05$) than baseline (Study Period) measurements (Table 1). However, no differences ($p>0.05$) were observed in fingernail cortisol levels during the Exam Period and the Fighting Period conditions. Mean cortisol level in toenails of subjects exposed to both physical stress (Fighting Period) and mental stress (Exam Period) conditions were similar to fingernail cortisol levels (Table 1).

Facial hair cortisol showed similar values with nail cortisol levels (Table 1). A higher correlation in cortisol levels was observed between facial hair samples and toenails ($r=0.73$) than between fingernails and toenails ($r=0.61$). Overall, cortisol showed significant correlations between fingernails and toenails ($p=0.001$, $r=0.61$), fingernails and facial hair ($p=0.01$, $r=0.54$), and toenails and facial hair ($p=0.001$, $r=0.73$).

	Cortisol level (pg/mg)			
Tissue	Baseline	Exam Period	Fighting Period	P value
Facial hair	71.2 ± 8.1^{b}	115.5 ± 10.8^{a}	110.2 ± 9.1^{a}	0.045
Fingernail	64.4 ± 8.5^{b}	94.3 ± 15.0^{a}	101.2 ± 13.9^{a}	0.043
Toenail	57.4 ± 12.5^{b}	103.2 ± 16.5^{a}	89.7 ± 16.0^{a}	0.031

Table 1: Cortisol levels in facial hair, fingernail and toenail of subjects during Exam Period (mental stress) and Fighting Period (physical stress) compared to baseline.

Discussion

As shown in this study, intensive physical-stress leads to higher levels of cortisol in body endpoints (facial hair and nails) with high correlations between different sample types. Intensive physical training, as a potent physical stressor, is known to be associated with an activation of the HPA axis and a subsequent release of cortisol to the blood stream [12]. In this study intense physical stress was generated in subjects during the Fighting Period. This is in agreement with another study reporting elevated cortisol levels in hair in endurance athletes over several months during a competition period [13].

Measuring cortisol levels in saliva, blood and urine for short term HPA activity can be problematic due to fluctuations in cortisol levels [6,14,15]. Collecting blood is invasive and may be anxiogenic, while urine collection takes a twenty-four hour period. Saliva sampling is also problematic due to the influence of food ingredients or medications which may contaminate results. Cortisol extraction from hair (facial hair in particular) and nails (finger and toe) may eliminate these issues and can be validated as a reliable means of assessment [6,8,15].

Hair growth rate is reported to be between 0.2-1.12 mm/day [16]. However, hair growth rates differ between races (Caucasian vs. Asian), gender, and age [16]. Moreover, in this study East Asian subjects (not having facial hair or very little growth) could not be employed for sampling. Since the aim of this study was to evaluate the cumulative cortisol level in the same subjects using their nails clippings (finger and toe) and facial hair (beard), only males were recruited. Previous experimental studies [14,17,18] established the hair follicle as an independent source of cortisol. However, two recent studies [6,8] reported the feasibility of cortisol extraction from fingernails as well. Initially, the physiological changes induced by the stress response serve an adaptive role as the body attempts to maintain homeostasis in spite of the stressor. However, a sustained increase in allostatic load is associated with a host of detrimental consequences. These may include the development or exacerbation of mental health disorders [19-22] hypertension [23], an increased risk for cardiovascular disease [24,25], obesity [26], type 2 diabetes [27], exacerbation of chronic obstructive pulmonary disease [28], asthma [29], exacerbation of skin conditions such as psoriasis [30], an increased risk of ulcerative colitis [31], reduced fertility [32] and poor gestational health [33].

The data of this study (Table 1) revealed that elevated cortisol levels in a variety of samples (facial, hair, and nails) were highly correlated. The similar ways cortisol is incorporated into new cells in nails and facial hair could explain the correlation between different sample types. Higher cortisol levels in subjects during physical and mental stress (Table 1) indicate that intensive physical training could have negative effects similar to chronic mental stress whereas moderate training is generally thought to be healthy and immunoprotective [34]. Skoluda et al. [13] reported that higher cortisol in the hair of endurance athletes than control subjects and occasional athletes. These higher cortisol levels found in subjects exposed to stress conditions do not necessarily show that stress conditions depress health. In fact, some levels of stress, known as eustress (good stress), are desirable for optimal performance and well-being [35].

The aim of this study was to preface another non-invasive cortisol measurement method (facial hair and toenail) followed up with fingernails [6,8] as this measurement method confirmed a previous study by the authors about cortisol in the teeth [36]. In this subject, cortisol levels in saliva were not examined, as we opted to test the validity of non-invasive methods instead, via means of facial hair, finger and toenails. Therefore, salivary cortisol measurements, as invasive cortisol measurement, were not taken daily during the ten day experimental periods. This may be investigated in future research. A limitation of this study was that subjects were limited to male participants. Future research can be done in both genders where facial hair is not included. The question if nail (finger and toe) cortisol levels and facial hair cortisol can be used as a retrospective stress assessment

during a given period are addressed by the results of the present study. These methods can be confirmed with further research.

Conclusion

This study is the first of its kind- to investigate cumulative cortisol levels within facial hair. The authors conclude that physical stress (such as that caused by intensive physical training), as well as mental stress (such as that which is related to exams), is associated with elevated cortisol levels in nails and facial hair. The data of this study shows the feasibility of facial hair cortisol assessment and the correlation of cortisol levels within body end-point sample types over short periods of time. The findings of this study should be investigated in further research regarding stable preservation of cortisol extraction, validation of the assessment, and expansion of knowledge of the criteria. More research is needed in order to draw a conclusion regarding the relationship between facial hair cortisol and nail cortisol and their possible relationship with various types of health disorders.

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Disclosure

The author reports no conflicts of interest in this work.

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